

AMENDMENTS TO THE SPECIFICATION

To replace SEQ ID NOs 2-4 on pages 3-4, please replace paragraph [0011] with the following:

--- In one aspect of the invention, the application of active peptides of the invention result in stabilization of microfilaments and cause cross-linking of F-actin *in vitro* and *in situ*. The peptides of the present invention, along with inactive control peptides, may be characterized as follows, where the conventional single letter amino acid code letters are used and the derivative source of the peptide is indicated:

SEQ ID NO: 1	ENGIVRKWISRFEVW	Consensus active SuSy peptide
SEQ ID NO: 2	ENGILRKWISRFDVW PYL	<i>Zea mays</i> SuSy1 367-381
SEQ ID NO: 3	ENGIVRKWISRFEVW PYL	<i>Zea mays</i> SuSy2 375-389
SEQ ID NO: 4	ENGILKKWISRFDVW PYL	<i>Zea mays</i> SuSy3
SEQ ID NO: 5	EHGIVTNWDDMEKIW	<i>Drosophila. melanogaster</i> Actin 2; <i>Homo sapiens</i> β and γ Actin
SEQ ID NO: 6	EHGIITNWDDMEKIW	<i>Drosophila melanogaster</i> Actin 3, 5, 6; <i>Homo sapiens</i> α Actin
SEQ ID NO: 7	EHGIVKDWNDMERIW	<i>Drosophila melanogaster</i> ARP1
SEQ ID NO: 8	ENGVVRNWDDMCHVW	<i>Drosophila melanogaster</i> ARP2
SEQ ID NO: 9	GDRVLSRLHSVRERIGK	SS1 inactive Control peptide
SEQ ID NO: 10	GIVRKWISRFEVWPYLKK	SS2 active peptide SuSy 377-392
SEQ ID NO: 11	ILRVPFRTENGIVRK	SS11 inactive peptide
SEQ ID NO: 12	GIVRKWISRFEVWPYL	SS12 active synthetic peptide
SEQ ID NO: 13	GIVRKAISRFEVAPYL	SS15 less active synthetic peptide
SEQ ID NO: 14	SRFEVWPYL	SS16 less active synthetic peptide
SEQ ID NO: 15	GPTLKRTASTAFMNTTSKK	NR11 inactive synthetic peptide
SEQ ID NO: 16	GRMRRIATVEMMKK	SP26 inactive synthetic peptide
SEQ ID NO: 17	WISRFEVW	SMIN less active synthetic peptide

SEQ ID NO: 18 RRISSVEDKK SP3 inactive synthetic peptide

SEQ ID NO: 19 EHGIVTNWDDMEKIWHHTFY Actin consensus sequence

SEQ ID NO: 20 EHGVRDWNDEMERIW *Homo sapiens* ARP1

SEQ ID NO: 21 ENGIVRNWDDMKHLW *Homo sapiens* ARP2

SEQ ID NO: 22 SRFEVW Core minimum SS synthetic peptide A

SEQ ID NO: 23 WISRFEVWPYLKK SS synthetic peptide B

SEQ ID NO: 24 ENGIVRKWISRFEVWPYLKK SS synthetic peptide C

Please replace paragraph [0013] with the following :

In one aspect, this activity may be conferred to such compounds by the presence of a shared motif described by the invention, and exemplified by the consensus sequence, Gly-Ile-X₁-X₂-X₃-Trp-X₄-X₅-X₆-X₇-X₈-X₁-Trp (SEQ ID NO: 29), where X₁ is a Val or other conservative substitution therefore, where X₂ is an Arg or other conservative substitution therefore, and X₃ to X₈ is any amino acid.

To replace the last row in Table 1 on page 11 with the following row to add the sequence identifier SEQ ID NO:25, please replace Table 1 with the following:

SEQ ID NO	Protein	Species	Position	Sequence
SEQ ID NO:1	SuSy consensus	Zea mays		ENGIVRKWISRFEVW
SEQ ID NO:2	SuSy 1	Zea mays	367-381	ENGILRKWISRFDVWPYL
SEQ ID NO:3	SuSy 2	Zea mays	375-389	ENGIVRKWISRFEVWPYL
SEQ ID NO:4	SuSy 3	Zea mays		ENGILKKWISRFDVWPYL
SEQ ID NO:5	Actin 2; $\beta\gamma$ Actin	Dro me; H. sapiens	73 - 87; 72 - 86	EHGIVTNWDDMEKIW

SEQ ID NO:6	Actin 3,5,6; α Actin	Dro me; H. sapiens	73 -- 87; 74 - 88	EHGIITNWDDMEKIW
SEQ ID NO:19	Actin consensus			EHGIVTNWDDMEKIWHHTFY
SEQ ID NO:7	ARP1	Dro me	76 -- 90	EHGIVKDWNDMERIW
SEQ ID NO:8	ARP2	Dro me	75 -- 89	ENGIVRNWDDMCHVW
SEQ ID NO:20	ARP1	H. sapiens	76 - 90	EHGVVRDWNDMERIW
SEQ ID NO:21	ARP2	H. sapiens	80 - 94	ENGIVRNWDDMKHLW
<u>SEQ ID NO:25</u>			CONSENSUS	E+GI+-W-----+W-----

Please replace paragraph [0026] with the following:

--- **Figure 3** is a series of ~~eolox~~ photographs of uninjected *Xenopus* blastomeres (Fig. 3A), upon injection of inactive peptides (Fig.3B), and active peptide (Fig. 3C), and microphotographs of the blastomere cleavage furrows with actin stained with rhodamine-phalloidin in uninjected normal embryos (Fig. 3D), and in embryos injected with the inactive peptide (Fig. 3E) and with the active peptide (Fig. 3F). ---

Please replace paragraphs [0029] and [0030] with the following:

--- **Figures 6A-6F** are ~~eolox~~ photographs of electrophoretic gels of fractions after the addition of the peptides to *in vitro* actin to determine the actin bundling activity of the peptides.

Figure 7 is a ~~eolox~~ photograph of stained gels of G-actin and F-actin showing that the bundling activity of SS2 is not affected by the addition of phalloidin.---

Please replace paragraph [0049] with the following:

---Thus peptides can be made, having the sequence of E-GI*---W-----W, (SEQ ID NO:26) where, I* means I or V, “-” means any amino acid, wherein the peptide causes actin bundling and inhibits actin depolymerization when polymerized in vitro with actin. In another embodiment, a peptide can be made having the sequence, EH*GIV*R*-W-----V*W (SEQ ID NO: 27), where H* means H or a conservative substitution therefore, V* means V or a conservative substitution therefore, and R* means R or a conservative substitution therefore, and - means any amino acid, wherein said peptide causes actin bundling and inhibits actin depolymerization when polymerized in vitro with actin.---

Please replace paragraph [0056] with the following:

--- In one embodiment, such peptides are created substantially homologous to the consensus sequence of Table 1. Effective peptides made using this formula should cause *in vitro* F-actin bundling and block actin depolymerization at peptide to actin ratios at least 100:1, more preferably 50:1, even more preferably about 20:1, more preferably 10:1, and most preferably at least 1:1. As shown in later examples, the first two residues in the Table consensus sequence may not be fully necessary for full activity. Therefore, in another embodiment, the peptides are created substantially homologous to a consensus sequence having the formula of formula (II): Gly-Ile-X₁-X₂-X₃-Trp-X₄-X₅-X₆-X₇-X₈-X₁-Trp (SEQ ID NO:29), where X₁ is Val or a conservative substitution therefore, X₂ is Arg or a conservative substitution therefore, and X₃ to X₈ can be any amino acid.---

Please replace the entire of paragraph [0057] with the following:

--- In another embodiment, active peptides can be fashioned using the formula (I) comprising: Gly-Ile-X₁-X₂-X₃-Trp-X₄-X₅-X₆-X₇-X₈-X₉-Trp-X₁₀-X₁₁-X₁₂ (SEQ ID NO:28) or a pharmaceutically acceptable salt thereof. In a preferred embodiment, the addition of a compound of formula (I) results in about 50% of bundled actin when polymerized in vitro with actin. In such an embodiment, each residue of the formula may be as follows:

X₁ = Ile, Val, or Leu

X₂ = Arg, Lys, Asn, or Thr

X₃ = Arg, Lys, Asn, or Asp

X₄ = Ile, Asp, Asn, or Glu

X₅ = Ser, or Asp

X₆ = Arg, Met, or Ala

X₇ = Phe, or Glu

X₈ = Asp, Glu, Lys, Arg, or His

X₉ = Val, or Ile

X₁₀ = Pro, or His

X₁₁ = Tyr, or His

X₁₂ = Leu, or Thr ---

Please replace paragraph [0061] with the following:

---Thus the invention further provides for a strategy of building active peptides based upon core sequences having minimal actin bundling activity. Active peptides can be made from discrete blocks of sequence from native sucrose synthase proteins, actin proteins or actin-related proteins, wherein the core blocks of sequence have substantial homology to the consensus sequence of Table 1. In such embodiments, if extended beyond the core sequence, the peptide can be extended using the corresponding amino acid sequence of a native sequence such as the *Zea mays* sucrose synthase protein or a human actin protein or actin-related protein. For example, based on the in vitro bundling activities of SEQ ID NO: 14 and SEQ ID NO: 17, SEQ ID NO: 22 can be seen as the basic core peptide from which a fully active synthetic peptide can be built upon, in order to create an active peptide such as SEQ ID NO: 10 or 12. Such a strategy of ~~slowly slowing~~ building peptides from smaller core blocks of sequence may be useful in cases where a smaller peptide is required, but the actin bundling activity must be retained.---

Please replace Table 3 on page 16 with the following:

SEQ ID NO.	synthetic peptide	Sequence	<i>In vitro</i> actin bundling activity
SEQ ID NO:22		SRFEVW	
SEQ ID NO:17	SMIN	WISRFEVW	less active
SEQ ID NO:14	SS16	SRFEVWPYL	less active
SEQ ID NO:23		WISRFEVWPYLKK	
SEQ ID NO:12	SS12	GIVRKWISRFEVWPYL	active
SEQ ID NO:10	SS2	GIVRKWISRFEVWPYLKK	active
SEQ ID NO:24		ENGIVRKWISRFEVWPYLKK	

Please replace paragraph [0088] with the following:

--Referring now to Fig. 1, use of BLASTP 2.0.8 revealed high homology between Sucrose Synthase sequence (SS2) with actin itself and actin-associating proteins. Fig. 1 shows that *Zea mays* Sucrose Synthase 2 (SUS2) residues 375-389 (SEQ ID NO: 3) have significant homology with *Zea mays* Sucrose Synthase 1 (SUS1) at residues 367-381 (SEQ ID NO: 2), with an expectancy score of 4e-04, 86% identical and 99% positively aligned. SUS2 has an expectancy score of 19, with 40% identity and 60% positives when aligned with residues 54-68 of *Zea mays* Actin (GenBank Accession No.: 1498382 (U60507)). Figure 1 further shows a sequence similarity between the SUS1 and SUS2 sequences and a consensus sequence of various actin proteins. This indicates the presently recognized possibility of a binding site on actin for a peptide having sequence similarities in this region. Shown below the Actin consensus sequence are various synthetic peptides made according to the teachings of the present specification and the activity of those peptides. As discussed below, the most active peptides, SS2 and SS12 contained the underlined subsequence GIVRWKI (SEQ ID NO:30), which appears to be necessary, but not sufficient, for activity.--

Please replace paragraphs [0096] with the following:

Referring now to ~~color~~ photographs of Fig. 3 the peptides have a severely abnormal effect on the embryonic development of cells. Embryos from *Xenopus laevis* were injected with the inactive control peptide of SEQ ID NO: 9 and the active peptide of SEQ ID NO: 10. Fig. 3A shows normal, uninjected embryos at the 8-cell stage (only 4 blastomeres can be seen; the other 4 cells are directly beneath them.) The same concentration of the inactive peptide was injected into embryos and had no effect as shown in Fig. 3B. The embryos resemble the uninjected normal embryos of Fig. 3A

Please replace paragraph [0114] with the following:

--- Referring now to Fig. 68B, the gel shows the effect of SS2 on polymerized actin *in vitro* with molar ratio of 0, 0.5, 1, 2 and 10. Purified rabbit muscle actin (2 nmol) was polymerized with SS2 *in vitro* for 30 min (G-actin, lanes 2 and 3) and subsequently incubated with different concentrations of SS2-peptide (0, 1, 2, 4 and 20 nmol) for another 30 min to make F-actin (lanes 4-8). Only the highest concentration of SS2 caused bundling of filamentous actin (lane 8). When SS2 was present during polymerization (G-actin, lanes 2, 3), it caused bundled actin at a molar ratio of peptide: actin of 2.---

Please replace paragraphs [00117] to [00118] with the following:

--- Fig. ~~8E6E~~ is a photo of gel showing the bundling activity of SS15, SS2 and SS16 peptides *in vitro* after addition to unpolymerized actin with increased molar ratio of peptide:actin = 10:1 and 100:1. At a molar ratio of 10 and 100, the polymerised actin is predominantly in the bundled form, with only small amounts of free F-actin after the addition of SS15 and SS16. Notice in lane 6, where the SS12 active peptide was added at a molar ratio of 100:1, there is clearly no band of free F-actin and very small bands at the lower molar ratio of 10:1, showing that the most effective peptide in bundling all the actin is likely SS12. The SS15 peptide does

not completely bundle all the actin until administered at a higher molar ratio than 10. The SS16 peptide however appears to exhibit a high activity in binding actin at a molar ratio of 10 and then reaches a maximum level of activity somewhere between a molar ratio of 10 and 100, after which the activity drops off dramatically.

Referring now to Fig. ~~8F6F~~8F6E, the gel shows bundling activity of SS12 at molar ratio to actin of 10 and 50. Even at the upper levels of a molar ratio of 50, all of the actin is in the bundled form with no G-actin or free soluble F-actin. The effective peptide to actin ratio for bundling for SS16 was >16:1 and for SS15, about 16:1, as opposed to 1:1 for SS2 and SS12, the most preferred embodiments.---